CHICAGO
BIOMEDICAL
CONSORTIUM

CBC Catalyst Award Proposal Title Page

Title of the proposal *(no more than 100 characters including white space)*

Improving Triple-Negative Breast Cancer Therapy by Targeting the Tumor Metabolic Microenvironment

Name, degree, title, institution, and contact information including the email address of each PI

Jonathan Coloff, PhD
Assistant Professor
Department of Physiology and Biophysics
University of Illinois Cancer Center
University of Illinois at Chicago
(312)996-7992
coloff@uic.edu

Alexander Muir PhD
Assistant Professor
Ben May Department of Cancer Research
University of Chicago
(773)834-6506
amuir@uchicago.edu

Do you have any current or pending grant applications that potentially overlap with this application? Check ONE:

YES ☐ **NO** ☒

If YES, please identify them.

Explain the overlap in the Biosketch section.

Does the proposed research involve animal subjects?	**Check ONE:**	**YES** ☒	**NO** ☐
Does the proposed research involve human subjects?	**Check ONE:**	**YES** ☐	**NO** ☒
Does the proposed research involve embryonic stem cells?	**Check ONE:**	**YES** ☐	**NO** ☒

(Note: if the data entered exceeds one page, it is acceptable to submit it as two pages.)

LAY SUMMARY: TNBC patients receive chemotherapy prior to surgery, and whether or not this treatment eradicates the tumor is among the best predictors of patient outcome. Thus, improving existing treatments or identifying more effective chemotherapies will improve TNBC patient outcome. Our collaborative team has made two discoveries that offer a new angle toward improving chemotherapy in TNBC. ***First***, the Coloff Lab has found that nutrient availability dramatically alters how TNBC cells respond to chemotherapies. ***Second***, the Muir Lab has developed tools to measure nutrient levels in tumor microenvironments (TME), which they have found to be unique compared to normal tissues. We propose to merge these two discoveries to define nutrient availability in TNBC tumors and determine how this unique nutrient environment affects the response to therapeutic agents. This work has the potential to lead to rapid improvements in patient outcome by identifying therapies that are more effective in the TNBC nutrient TME and opportunities to improve existing therapies by altering tumor microenvironment nutrient levels.

INNOVATION AND IMPACT STATEMENT: The current paradigm in cancer drug discovery assumes drug response is primarily determined by cell-intrinsic factors[1]. However, recent work from ourselves[2] and others[3-5] has found that therapeutic response of cancer cells is also driven by cell-extrinsic factors such as nutrient availability[6]. By quantifying nutrient levels in the TNBC TME and determining how this unique nutrient environment impacts chemotherapeutic response, our work will expand our understanding of TNBC biology and could revolutionize TNBC treatment. Despite already showing that physiological nutrient levels dramatically change how TNBC cells respond to therapy (Fig. 1) and that the nutrients found in the TNBC TME are truly unique (Fig. 2), we believe that this proposal would likely be considered too high-risk to be funded by conventional sources. Nevertheless, with this risk comes the potential for great rewards, and we are confident that an investment from the CBC will allow our labs to identify nutrient:drug interactions that will not only lead to new external funding opportunities, but will also generate intellectual property that could be leveraged to improve TNBC therapy.

PROPOSED RESEARCH: Using the unique strengths of our laboratories, we will test the novel ***hypothesis*** that abnormal nutrient availability in the TNBC TME significantly affects the response of TNBC to therapeutic agents.

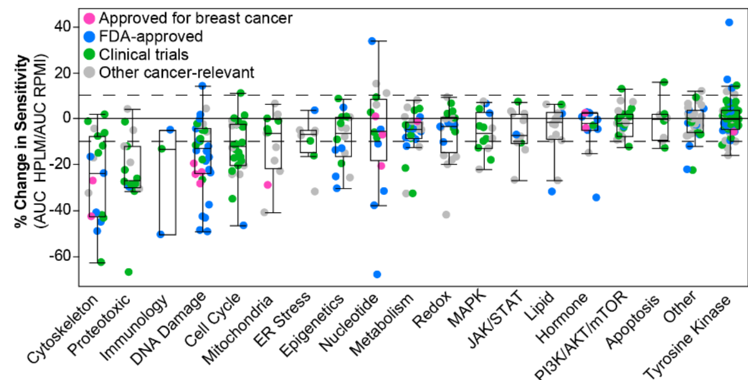

Figure 1: A) Differential sensitivity of anti-cancer compounds in TNBC cells (SUM149) growing in human plasma like medium (HPLM) relative to RPMI.

Aim 1: Define and model the nutrient microenvironment in breast tumors. We (Fig. 1) and others[6] have found that nutrient availability is a key regulator of cancer cell response to certain therapies. Thus, acquiring a molecular understanding of how the unique nutrient microenvironment found in TNBC tumors impacts the response of TNBC cells to chemotherapeutics will facilitate the development of maximally efficacious therapeutic regimens. In this aim, we will first profile the nutrient microenvironment of TNBC tumors (Aim 1A). We then use this information to build a culture model where TNBC cells can be grown while fed tumor-specific levels of >100 major nutrients (Aim 1B). This work will provide an atlas of nutrients in the TNBC TME and generate an experimentally tractable *ex vivo* system enabling studies in Aim 2 that will molecularly define how the nutrient TME of TNBC impacts chemotherapeutic response.

Aim 1A: Use quantitative metabolomics of tumor interstitial fluid to define the TNBC nutrient microenvironment. We will use the TME nutrient profiling pipeline that the Muir lab has developed[7] to map nutrient availability in the tumor interstitial fluid (TIF; the perfusate of solid tumors) of TNBC. While these studies would ideally be performed on TIF from patient samples, through our conversations with the UI Health breast oncology team it is clear that there are several obstacles that make this impractical. First, nearly all TNBC patients receive neoadjuvant chemotherapy prior to surgery, making the availability of treatment-naïve surgical samples very limited. Second, core biopsy samples are not large enough to yield sufficient quantities of TIF for metabolomic analysis. Third, because circulation is cut off the moment the tumor tissue is resected,

it is highly likely that nutrient levels are changed during the ~30 minutes that this still metabolically active tissue is examined by the pathology team. Due to these issues, we believe that mouse models of TNBC will provide a more accurate representation of nutrients in human TNBC tumors than patient samples. Therefore, in these studies we will utilize TIF isolated from the *MMTV-Cre; Brca1$^{F22-24/F22-24}$; Trp53$^{fl/+}$* (BRCA/p53) genetically engineered mouse model (GEMM) of TNBC because it features mutations commonly occurring in human TNBC and leads to the formation of receptor negative tumors similar to human basal-like TNBC in the context of a normal immune system. This TNBC model is generally regarded as the model that most accurately recapitulate human TNBC[8-10]. Of particular importance to our proposed study, this model is widely used in the study of TNBC therapy response as it faithfully recapitulates patient responses to therapy[8-10]. In addition to profiling metabolites, we will also measure TIF albumin and electrolyte concentrations. These experiments will provide a quantitative atlas of major nutrients and electrolytes found in tumors, providing new insight into the metabolic TME of TNBC.

Aim 1B: Develop a custom TNBC culture model that replicates the TNBC nutrient microenvironment. Using the quantitative measurements from Aim 1A we will build a cell culture medium containing TNBC-specific levels of nutrients, albumin, and electrolytes, similar to that which we have previously built based on pancreatic TIF concentrations, which improved the fidelity of *ex vivo* pancreatic cancer models to *in vivo* biology[11]. Nutrients will be chosen based on: (1) commercial availability at high purity, (2) stability in aqueous solution, and (3) presence in TNBC TIF at a concentration > 0.5uM. Dry powders will be compounded using a knife mill homogenizer in separate pools in order to allow rapid identification of bio-active metabolites (Aim 2B). Our medium, termed breast tumor interstitial fluid medium (bTIFm), will be reconstituted with 10% dialyzed FBS and we will use LC-MS metabolomics to ensure bTIFm reliably matches our desired formulation.

Expected Results, Pitfalls, and Alternative Approaches: We have performed a pilot on isolated TIF from BRCA/p53 tumors, which revealed dramatic differences in nutrient availability in TNBC (Fig 2). As such, we do not anticipate significant pitfalls in murine TIF analysis. A potential pitfall is that our murine model may not fully recapitulate human TNBC nutrition. To assess this possibility, will utilize human-derived PDX models of TNBC (available in the Coloff Lab) to survey potential human-specific TME nutrients. Use of hypoxia chambers and/or 3D cultures could also improve the fidelity of our *in vitro* TNBC model.

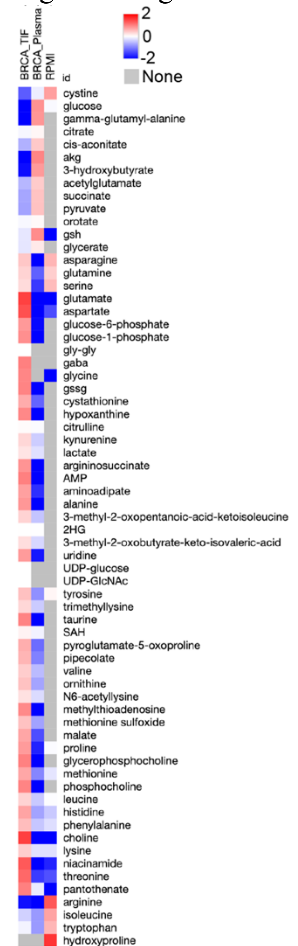

Figure 2: Relative levels of indicated nutrients in BRCA TNBC TIF, paired plasma and standard culture medium.

<u>Aim 2: Determine the impact of breast tumor nutrient availability on drug response.</u> Our data demonstrate that efficacy of experimental and standard-of-care therapies for TNBC are impacted by nutrient availability (Figs. 1 & [11]). Thus, there may be existing drugs whose efficacy is potentiated by the nutrient TME or nutrient:drug interactions that limit therapeutic efficacy. In this aim, we will identify drugs that are impacted by the TNBC nutrient TME (Aim 2A) and the mechanisms by which they do so to provide molecular insight into therapeutically relevant nutrient:drug interactions (Aim 2B).

Aim 2A: Profile the impact of bTIFm on TNBC response to therapeutic agents. To identify TNBC therapies whose efficacy is driven by the nutrient TME, we will perform a drug screen using TNBC cells cultured in either bTIFm or RPMI in collaboration with Dr. Isaac Harris at the University of Rochester (see letter of support), who has established a screening platform containing over 600 FDA-approved or clinical tested anti-cancer compounds[12-16] and who performed our HPLM study (Fig. 1). This screen will use SUM149 TNBC cells which recapitulate the genetics of our murine model and are optimized for screening with this platform. Hits from this screen will be validated in four TNBC cell lines of varying genotypes.

Aim 2B: Identify the nutrient(s) and alterations in cellular processes responsible for differential drug responses. For drugs whose effect on TNBC is modulated by the nutrient TME, we will first identify which

nutrient(s) are responsible for impacting efficacy. As mentioned, bTIFm will be constructed using 8 separate pools that are readily swappable with RPMI pools. Once the efficacy-altering pools are identified, nutrients will be broken down into sub-pools until individual nutrients impacting drug efficacy are identified. After identifying nutrient(s) responsible for altering drug efficacy, we will identify mechanisms by which nutrients alter drug response by profiling TNBC cells cultured in bTIFm and RPMI using metabolomics, metabolic flux analyses, RNA sequencing, and reverse phase protein array. Both the Coloff and Muir labs have previously used similar methods to identify mechanisms of drug:nutrient interactions and to develop interventions that will potential drug response (see **IMPLEMENTING OUR FINDINGS** discussion below).

Expected Results, Pitfalls, and Alternative Approaches: Both the Coloff and Muir labs have experience identifying nutrient:drug interactions and we are confident that the proposed experiments can be performed during the two year award period. Given the drastic differences in nutrient levels observed in TIF from TNBC tumors (Fig. 2) and our results that plasma-like nutrient levels dramatically alter therapeutic response (Fig. 1), we expect that there will be significant differences in sensitivity to numerous therapeutics. In our experience most of the drug:nutrient interactions can be traced back to one nutrient, but our approach is also suited to delineate interactions between multiple nutrients should we identify more complex interactions. A weakness of our approach is that our screen will not evaluate the impact of bTIFm on immune checkpoint inhibitors (which have recently been approved for TNBC[17]) or others that may impact tumor growth by affecting stromal cell populations.

IMPLEMENTING OUR FINDINGS TO IMPROVE PATIENT OUTCOME: A likely outcome of this project will be the development of therapeutic interventions to manipulate tumor nutrient levels to improve drug efficacy. An example of this can be found in our drug screen using human plasma-like medium (Fig. 1), where we found that the activity of the experimental drug rigosertib is strongly inhibited by uric acid, which is found in physiological media and *in vivo*, but not traditional culture media. Rigosertib has worked well in laboratory studies but failed in clinical trials. Our data suggests that the elevated uric acid levels found *in vivo* may underlie its failure in the clinic. We are currently working with the UIC Office of Technology Management and Onconova (who hold the patent for rigosertib) to determine if commonly used therapies to lower systemic uric acid levels for treatment of gout and tumor lysis syndrome, including XDH inhibitors and uric acid degrading enzymes, may be effective in combination with rigosertib.

A second likely outcome is the discovery of drugs potentiated by TME nutrients that have previously been overlooked as potential TNBC therapies due to lack of efficacy in preclinical screening. As the drugs we are screening are clinically used/tested, we anticipate TME-potentiated drugs can be translated for TNBC therapy.

NATURE OF INTERINSTITUTIONAL COLLABORATION: The expertise of both groups is required for this undertaking. The Muir Lab brings expertise in TIF isolation, quantitative metabolomics, and media formulation, while the Coloff Lab brings expertise in breast cancer biology, TNBC models, and studies of TNBC therapeutic sensitivity. The design of this project arose from discussion between Dr. Muir and Dr. Coloff over several years and builds on the strengths of each lab.

CRITERIA FOR MEASURING PROJECT SUCCESS: Success of this project will be measured by three milestones. The first milestone will be successful generation of bTIFm. The second milestone will be the identification of cancer therapies that are affected by the unique nutrients found in the TNBC TME. The third will be mechanistic understanding of how several of the identified therapies are altered by TNBC nutrition.

LONG-TERM FUNDING PLAN: The CBC catalyst award will provide us with funding needed to generate proof-of-concept data that challenges the drug discovery paradigm and will identify novel TNBC drugs and approaches to improve existing TNBC treatment efficacy. Given the high impact of these findings, we believe that with specific drug:nutrient interactions in hand we will be in the position to apply for two additional funding mechanisms to move this proof-of-concept work towards translational application. First, we anticipate submission of a co-PI R01 application to the NCI on the core findings of this work. Second, we will prepare applications to foundations such as the American Cancer Society and the American Association of Cancer Research. We anticipate validating drug:nutrient interactions half-way through the second year of this award, which will lead to submission of additional applications by the end of the two-year CBC funding period.

REFERENCES

1 Horvath, P. *et al.* Screening out irrelevant cell-based models of disease. *Nat Rev Drug Discov* **15**, 751-769 (2016). https://doi.org:10.1038/nrd.2016.175

2 Muir, A. *et al.* Environmental cystine drives glutamine anaplerosis and sensitizes cancer cells to glutaminase inhibition. *Elife* **6** (2017). https://doi.org:10.7554/eLife.27713

3 Jin, M. Z. & Jin, W. L. The updated landscape of tumor microenvironment and drug repurposing. *Signal Transduct Target Ther* **5**, 166 (2020). https://doi.org:10.1038/s41392-020-00280-x

4 Fiedler, E. C. & Hemann, M. T. Aiding and Abetting: How the Tumor Microenvironment Protects Cancer from Chemotherapy. *Annual Review of Cancer Biology, Vol 3* **3**, 409-428 (2019). https://doi.org:10.1146/annurev-cancerbio-030518-055524

5 Hirata, E. & Sahai, E. Tumor Microenvironment and Differential Responses to Therapy. *Cold Spring Harb Perspect Med* **7** (2017). https://doi.org:10.1101/cshperspect.a026781

6 Muir, A. & Vander Heiden, M. G. The nutrient environment affects therapy. *Science* **360**, 962-963 (2018). https://doi.org:10.1126/science.aar5986

7 Sullivan, M. R. *et al.* Quantification of microenvironmental metabolites in murine cancers reveals determinants of tumor nutrient availability. *Elife* **8** (2019). https://doi.org:10.7554/eLife.44235

8 McCarthy, A. *et al.* A mouse model of basal-like breast carcinoma with metaplastic elements. *J Pathol* **211**, 389-398 (2007). https://doi.org:10.1002/path.2124

9 Reis-Filho, J. S. & Tutt, A. N. Triple negative tumours: a critical review. *Histopathology* **52**, 108-118 (2008). https://doi.org:10.1111/j.1365-2559.2007.02889.x

10 Holen, I., Speirs, V., Morrissey, B. & Blyth, K. In vivo models in breast cancer research: progress, challenges and future directions. *Dis Model Mech* **10**, 359-371 (2017). https://doi.org:10.1242/dmm.028274

11 Apiz-Saab, J. J. *et al.* Pancreatic tumors activate arginine biosynthesis to adapt to myeloid-driven amino acid stress. *bioRxiv*, 2022.2006.2021.497008 (2022). https://doi.org:10.1101/2022.06.21.497008

12 Harris, I. S. *et al.* Deubiquitinases Maintain Protein Homeostasis and Survival of Cancer Cells upon Glutathione Depletion. *Cell Metab* (2019). https://doi.org:10.1016/j.cmet.2019.01.020

13 Nicholson, H. E. *et al.* HIF-independent synthetic lethality between CDK4/6 inhibition and VHL loss across species. *Sci Signal* **12** (2019). https://doi.org:10.1126/scisignal.aay0482

14 Shu, S. *et al.* Synthetic Lethal and Resistance Interactions with BET Bromodomain Inhibitors in Triple-Negative Breast Cancer. *Mol Cell* **78**, 1096-1113 e1098 (2020). https://doi.org:10.1016/j.molcel.2020.04.027

15 Drijvers, J. M. *et al.* Pharmacologic Screening Identifies Metabolic Vulnerabilities of CD8(+) T Cells. *Cancer Immunol Res* **9**, 184-199 (2021). https://doi.org:10.1158/2326-6066.CIR-20-0384

16 Koduri, V. *et al.* Targeting oncoproteins with a positive selection assay for protein degraders. *Sci Adv* **7** (2021). https://doi.org:10.1126/sciadv.abd6263

17 Cortes, J. *et al.* Pembrolizumab plus chemotherapy versus placebo plus chemotherapy for previously untreated locally recurrent inoperable or metastatic triple-negative breast cancer (KEYNOTE-355): a randomised, placebo-controlled, double-blind, phase 3 clinical trial. *Lancet* **396**, 1817-1828 (2020). https://doi.org:10.1016/S0140-6736(20)32531-9

COLOFF LAB BUDGET JUSTIFICATION

Principal Investigator:

Name: **Jonathan Coloff, PhD**
Role: **Principal Investigator**
Effort: **0% 0 CM**

Dr. Coloff will direct the components of this proposal that are performed in his laboratory. He will also coordinate communication within the research team, supervise experiments and data analysis, and write manuscripts and progress reports.

Other Personnel:

Name: **Huiping Zhao**
Role: **Laboratory Technician**
Effort: **50% 6 CM per year**

Huiping Zhao is a technician in the Coloff Lab and will perform the mouse and tissue culture experiments described in this proposal. Huiping has extensive experience studying breast cancer including use of mouse models and tissue culture.

Mouse Costs: $5728
MMTV-Cre; Brca1$^{F22\text{-}24/F22\text{-}24}$; *Trp53*$^{fl/+}$ breeding pair: $1000 including shipping
Cage costs (8 cages x 8 months x $0.90/day): $1728
Genotyping costs: $1000
Reagents and disposables for TIF and serum isolation: $2000

Tissue Culture and General Supplies: $25,756
We will perform a large amount of tissue culture work with associated costs.

Pharmaceutical grade drugs for explant culture and screen follow up: $5,972
Tissue culture dishes, plates, sponges for explant culture, *etc*: $6,902
Disposables (gloves, tips, pipettes, *etc*): $5,855
bTIFm components (for media swapping, *etc*): $7,039

External RNA sequencing: $7,400

RNA prep: $1000
RNA sequencing and data processing with Novogene: $300/sample, $5,400 total
qPCR plates, reagents, instrument time for validation and follow-up: $1000

External Reverse Phase Protein Arrays: $9,900

Sample prep and RPPA analysis at MD Anderson RPPA Core facility: $300/sample, $5,400 total
Antibodies for validation and follow up western blots: $350/antibody, $3,500 total
Gels, buffers, other supplies for western blots: $1,000

Total Budget: $125,000

Coloff Lab Scope of Work:

The Coloff Lab will perform the following experiments as part of our collaborative project with the Muir Lab:

- Establish *MMTV-Cre; Brca1*$^{F22\text{-}24/F22\text{-}24}$; *Trp53*$^{fl/+}$ colony
- Isolate tumor interstitial fluid from tumor bearing mice
- Screen and explant hit validation
- Identify nutrient:drug interactions using pool-swapping approach
- Perform RNAseq on TNBC cells grown in RPMI and bTIFm
- Perform RPPAs on TNBC cells grown in RPMI and bTIFM

MUIR LAB BUDGET JUSTIFICATION

Principal Investigator:

Name: **Alexander Muir, PhD**
Role: **Principal Investigator**
Effort: **0% 0 CM**

Dr. Muir will direct all the proposed work to be performed in his group. He will be responsible for mentoring and supervising Grace Croley (see other personnel) who will perform many of the proposed experiments and he will assist with data analysis and interpretation. He will also coordinate work with the Coloff group and other members of the research team, write manuscripts and progress reports.

Other Personnel:

Name: **Grace Croley**
Role: **Laboratory Technician**
Effort: **50% 6 CM per year**

Grace Croley is a talented research technician in the Muir Laboratory with 1 year of experience studying cancer cell metabolism using mass spectrometry and TIF-based media. She will assist mass spectrometry analysis of samples and generation of TIF-based media.

Reagents and sera for producing bTIFm: $27,915 in year 1
15L of dialyzed fetal bovine serine for producing 75L of bTIFm and RPMI: $550/L x 15L = $16,500
Metabolite costs for producing 75L of bTIFm: $95.5/L x 75L = $7,165
Metabolite costs for producing 75L of RPMI: $24.6/L x 75L = $1,850
Albumin costs for producing 75L of bTIFm: $32/L x 75L = $2,400

Material for lactate dehydrogenase (LDH) and electrolyte analysis: $10,000 year 1
We will perform many LDH analyses in year 1 to ensure TIF sample purity and electrolyte analysis of TIF samples:
Kits for measurement of LDH, albumin and electrolytes: 10 analytes x $600/kit = $6,000
Disposables (gloves, serological pipettes, pipette tips, 96-well plates, etc.): $4,000

Mass spectrometry and cell culture consumable costs: $10,000 for year 1 and $10,000 for year 2
We will perform a large amount of tissue culture work and mass spectrometry sample preparation work:
Mass spec vials, solvents, isotopically labeled internal standards: $7,000/year in years 1/2, $13,526 in year 3
Tissue culture dishes, consumables, trypsin, filters for media etc.: $6,000/year
Laboratory consumables (serological pipettes, pipette tips, sample tubes, gloves, etc.): $7000/year

Core Services: $15,120 for year 1, $6,500 for year 2
We will extensively use the University of Chicago Metabolomics Platform with which we have a longstanding working relationship for metabolomic analysis:
Quantitative metabolomics of TNBC TIF in year 1: $60/sample x 132 samples including standards = $7,920
Quantitative metabolomics of bTIFm in year 1: $60/sample x 120 samples including standards = $7,200
Targeted/untargeted metabolomics and lipidomic analysis of TNBC samples in year 2: $225/sample x 20 samples with 40 hrs of analysis for untargeted and lipidomic samples at $50/hr = $6,500

Total Budget: $125,000

Muir Lab Scope of Work:

The Muir Lab will perform the following experiments as part of our collaboration with the Coloff Lab:

- Verify purity by LDH assay of TNBC interstitial fluid isolates from Coloff group
- Perform and analyze quantitative metabolomics of TNBC interstitial fluid isolates
- Analyze albumin and electrolyte concentrations in TNBC interstitial fluid isolates
- Generate bTIFm and use quantitative metabolomics to ensure correct formulation
- Perform metabolomics on TNBC cells cultured in standard conditions (RPMI) and bTIFm conditions and analyze resulting data

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: Jonathan Louis Coloff

eRA COMMONS USER NAME: JONATHAN_COLOFF

POSITION TITLE: Assistant Professor

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Iowa State University, Ames, IA	B.S.	05/2002	Biology
Duke University, Durham, NC	Ph.D.	10/2010	Pharmacology
Harvard Medical School, Boston, MA	Postdoc	06/2018	Cancer Biology

A. Personal Statement

The long-term goals of my lab are to understand how the unique metabolic phenotypes in cancer cells arise, and to discover approaches of targeting metabolism for cancer therapy. Key to achieving these goals is to have cancer models that facilitate experimentation while remaining faithful to human disease. While there has always been a bottleneck of promising *in vitro* cancer discoveries that turn out to be not relevant in real tumors, I remain a firm believer that tissue culture is not inherently inadequate and can be improved so as to better recapitulate the *in vivo* tumor environment. This has led me to explore use of alternative tissue culture systems, including 3D organotypic cultures and modern organoid models, and to adapt such models for studying cellular metabolism. More recently, work by several labs has highlighted the problematic nature of the non-physiological nutrient levels found in traditional tissue culture media and have demonstrated that culture systems that more accurately represent the nutrients found *in vivo* can correct many of the metabolic artifacts of traditional systems. This has led our group to make significant use of human plasma-like medium, which we have found to have profound effects on the behavior of cancer cells. Most notably, we have found that the therapeutic response of triple-negative breast cancer cells to a wide-variety of therapeutic agents is very strongly impacted by nutrient availability. While we are currently working on how nutrient availability can be modified *in vivo* to improve TNBC therapies, work from my colleague Alex Muir has clearly demonstrated that the nutrient levels found in actual tumors can vary dramatically from those found in plasma. This suggests that while our use of plasma-like media offers an improvement over traditional systems, to truly understand how nutrients impact therapeutic response in TNBC we must understand what nutrients are actually available to cancer cells within the tumor microenvironment. This has led to our current collaboration with the Muir Lab, which I believe will be among the most important work yet from my laboratory. Not only will we identify opportunities to improve the effectiveness of chemotherapy in TNBC, but the medium we develop could be a significant resource for the breast cancer research community, thereby increasing the potential long-term impact of this project.

B. Positions and Honors

Employment

2018 – Present Assistant Professor, *University of Illinois at Chicago*

2018 – Present	Full Member, *University of Illinois Cancer Center*
2010 – 2018	Postdoctoral Fellow, *Harvard Medical School*, Advisor: Joan Brugge
2005 – 2010	Graduate Student, *Duke University*, Advisor: Jeffrey Rathmell
2003 – 2005	Assistant Scientist, *University of Minnesota*, Advisor: Michel Sanders
2000 – 2003	Undergraduate Researcher, *Iowa State University*, Advisor: Janice Buss

Honors

2022	Philip L. Hawley Distinguished Faculty Award, UIC Physiology
2021	UIC College of Medicine Departmental Rising Star Award
2021 – Present	NCI R37 Method to Extend Research in Time Award
2018	UIC Breast Cancer Research Group Pilot Award
2016	Aspen Cancer Conference Fellow
2016	Scholarship Award, Keystone Symposia: New Frontiers in Understanding Tumor Metabolism
2015	Poster Prize Award, Dana Farber/Harvard Cancer Center Breast-Gynecologic Symposium
2015	Scholarship Award, Keystone Symposia: Integrating Metabolism and Tumor Biology
2013 – 2016	American Cancer Society Postdoctoral Fellowship
2012	Scholarship Award, Keystone Symposia: Cancer Metabolism
2011 – 2016	Organizer, Harvard Medical School Cancer Metabolism Group
2009	Citizen's Advisory Council Poster Prize Award, Duke Cancer Center Annual Meeting
2008	Outstanding Poster Presentation Award, Duke Cancer Center Annual Meeting
2008	Scholarship Award, Keystone Symposia: Cell Death in the Immune System
2001	National Science Foundation Research Experience for Undergraduates Award, Iowa State University

C. Contributions to Science

Aerobic Glycolysis Protects Activated Lymphocytes and Cancer Cells from Apoptosis

Activated lymphocytes and cancer cells both display high rates of aerobic glycolysis, but whether this unique metabolic phenotype impacts other aspects of cell biology was not clear. As a graduate student studying immunometabolism in Jeff Rathmell's laboratory, we hypothesized that the highly glycolytic metabolic phenotype observed upon lymphocyte activation and in most tumors (*i.e.*, the Warburg effect) contributes to the ability of cancer cells to evade apoptosis. Working with lymphocyte models of growth factor-dependent metabolism and survival, we tested this hypothesis using two methods: 1) by engineering cells to have a highly glycolytic metabolic program independent of any direct signaling effects, and 2) by disrupting glycolytic metabolism in cancer cells with an Akt-driven glycolytic phenotype. Both of these methods led to similar conclusions—that highly glycolytic cells were resistant to cell death due to metabolic suppression of the pro-apoptotic BH3-only protein Puma and activation of the anti-apoptotic Bcl-2 protein Mcl-1. This work demonstrated how the metabolic state of a cell can influence cell fate decisions, which is manifest in cancer cells by high rates of glycolysis promoting cell survival. Importantly, this was an early example of how the metabolic state of a cell can influence cell fate in human disease.

a. Zhao Y, **Coloff JL**, Ferguson EC, Jacobs SR, Cui K, Rathmell JC. Glucose metabolism attenuates p53 and Puma-dependent cell death upon growth factor deprivation. *Journal of Biological Chemistry*. 2008;283(52):36344-53. doi: 10.1074/jbc.M803580200. PMID: 18990690; PMCID: PMC2606014.
b. **Coloff JL**, Zhao Y, Rathmell JC. The Integration of Metabolism and Cell Death. In: Dong Z, Yin X, editors. Essentials of Apoptosis: Humana Press; 2009. p. 223-37.
c. **Coloff JL**, Mason EF, Altman BJ, Gerriets VA, Liu T, Nichols AN, Zhao Y, Wofford JA, Jacobs SR, Ilkayeva O, Garrison SP, Zambetti GP, Rathmell JC. Akt requires glucose metabolism to suppress puma expression and prevent apoptosis of leukemic T cells. *Journal of Biologicial Chemistry*. 2011;286(7):5921-33. doi: 10.1074/jbc.M110.179101. PMID: 21159778; PMCID: PMC3037705.
d. **Coloff JL***, Macintyre AN*, Nichols AG, Liu T, Gallo CA, Plas DR, Rathmell JC. Akt-dependent glucose metabolism promotes Mcl-1 synthesis to maintain cell survival and resistance to Bcl-2 inhibition. *Cancer Research*. 2011;71(15):5204-13. doi: 10.1158/0008-5472.CAN-10-4531. PMID: 21670080; PMCID: PMC3148426.

Extracellular Matrix Signaling Regulates Metabolism in Epithelial Cells

Like cytokines in lymphocytes, ECM cooperates with epithelial growth factors to mediate the transmission of cell signals that control cellular metabolism. Using gas chromatograph-mass spectrometry (GC-MS) and stable isotope tracers, we were able to identify a key role for ECM signaling in regulating pyruvate dehydrogenase flux, thereby controlling the flow of carbon into the TCA cycle. This work discovered mechanisms by which microenvironmental factors can influence metabolism and demonstrated how transformed epithelial cells survive in the absence of ECM contact. This work demonstrates the dynamic nature of tumor metabolism and how relevant metabolic pathways may change depending on the state the tumor cells are in (*i.e.*, growing outside of their normal environment, metastasizing, *etc*,).

a. Grassian AR*, Metallo CM*, **Coloff JL**, Stephanopoulos G, Brugge JS. Erk regulation of pyruvate dehydrogenase flux through PDK4 modulates cell proliferation. *Genes & Devevelopment*. 2011;25(16):1716-33. doi: 10.1101/gad.16771811. PubMed PMID: 21852536; PubMed Central PMCID: PMC3165936.
b. Grassian AR, **Coloff JL**, Brugge JS. Extracellular matrix regulation of metabolism and implications for tumorigenesis. Cold Spring Harbor Symposia on Quantitative Biology. 2011;76:313-24. doi: 10.1101/sqb.2011.76.010967. PMID: 22105806.
c. **Coloff JL**, Brugge JS. Coping with the metabolic stress of leaving home. *Cell Research*. 2016 Jul;26(7):757-8. doi: 10.1038/cr.2016.61. PMID: 27216116; PMCID: PMC5129879.
d. **Coloff JL**, Brugge JS. Metabolic changes promote rejection of oncogenic cells. *Nature Cell Biology*. 2017 Apr 27;19(5):414-415. PMID: 28446818

Proliferation and Tumor Lineage are Critical Determinants of the Cancer Metabolic Phenotype

Despite considerable progress in recent years, we still do not fully understand why tumors display unique metabolic phenotypes relative to normal tissues. A predominant hypothesis is that cancer cells need to alter their metabolism to support their aberrant proliferation rates. However, in order to understand whether there are truly unique aspects of tumor metabolism that might not be present in normal proliferating cells, it is necessary to first understand how normal cells regulate metabolism as they transition between proliferative and quiescent cell states. To this end, I developed and utilized a 3D tissue culture model to characterize the metabolic transition that occurs as mammary epithelial cells transition between periods of proliferation and quiescence. Of the many metabolic differences between proliferating and quiescent cells, changes in how cells utilize glutamate stood out. I found that proliferating cells preferentially utilize glutamate via transaminases to generate non-essential amino acids and couple carbon and nitrogen anabolic metabolism, while quiescent cells decoupled carbon and nitrogen metabolism by utilizing glutamate via glutamate dehydrogenase. An analysis of TCGA data revealed that most rapidly proliferating breast tumors used a similar program, suggesting that cancer cells hijack a normal cellular proliferation mechanism to metabolically support their aberrant proliferative program. Expanding on this work, I led a project where we performed a large-scale computational analysis of factors regulating metabolic gene expression in tumors. This analysis led to the surprising conclusion that the underlying genetic mutations in tumors do not impart any particular metabolic phenotype; rather, the rate of proliferation and tumor lineage (*i.e.*, cell- or tissue-of-origin) are the primary determinants of metabolic gene expression programs in cancer.

a. **Coloff JL**, Murphy JP, Braun CR, Harris IS, Shelton LM, Kami K, Gygi SP, Selfors LM, Brugge JS. Differential Glutamate Metabolism in Proliferating and Quiescent Mammary Epithelial Cells. *Cell Metabolism*. 2016; 23(5):867-80. doi: 10.1016/j.cmet.2016.03.016. PMID: 27133130.
b. **Coloff JL.** Glutamate Dehydrogenase to the Rescue. *Molecular Cell*. 2018 Jan 4;69(1):1-2. doi: 10.1016/j.molcel.2017.12.015. PMID: 29304329.
c. Stover DG, **Coloff JL**, Barry WT, Brugge JS, Winer EP, Selfors LM. The Role of Proliferation in Determining Response to Neoadjuvant Chemotherapy in Breast Cancer: A Gene Expression-Based Meta-Analysis. *Clinical Cancer Research*. 2016. doi: 10.1158/1078-0432.CCR-16-0471. PMID: 27330058. PMCID: PMC5161615.
d. Selfors LM, Stover DG, Harris IS, Brugge JS*, **Coloff JL***. Identification of cancer genes that are independent of dominant proliferation and lineage programs. *PNAS*. 2017 Dec 26;114(52):E11276-E11284. doi: 10.1073/pnas.1714877115. Epub 2017 Dec 11. PMID: 29229826.

Serine Auxotrophy as a Therapeutic Vulnerability in Luminal Breast Cancer

Breast tumors are made up of at least two primary lineages – basal and luminal – that are as different from each other as they are from tumors arising in distinct tissues. To identify lineage-dependent metabolic vulnerabilities in breast cancer, in my laboratory we have taken the approach of analyzing differences in metabolic gene expression between basal and luminal breast tumors where we have identified phosphoserine aminotransferase 1 (*PSAT1*) as the most differentially expressed metabolic gene, with luminal tumors expressing 26-fold less *PSAT1* than basal tumors. Low PSAT1 prevents luminal breast cancer cells from synthesizing serine *de novo* and makes them entirely dependent on exogenous serine for proliferation. Suppression of PSAT1 expression in luminal tumors is caused by lineage-specific hypermethylation of the *PSAT1* gene, which is unique to luminal breast tumors among all tumor types, suggesting that serine auxotrophy may be a luminal-specific metabolic vulnerability. Indeed, we have found that luminal breast tumors are highly sensitive to dietary serine starvation. Ongoing projects are aimed at identifying the transporter(s) responsible for serine uptake in luminal breast cancer cells and acquiring a better understanding of the physiological consequences of serine starvation.

- Choi BH, **Coloff JL**. The Diverse Functions of Non-Essential Amino Acids in Cancer. *Cancers*. 2019 May 15;11(5):675. doi: 10.3390/cancers11050675. PMID: 31096630. PMCID: PMC6562791.
- Choi BH, Rawat V, Hogstrom J, Burns PA, Conger KO, Ozgurses ME, Patel JM, Mehta TS, Warren A, Selfors LM, Muranen T**, Coloff JL**. Lineage-specific silencing of PSAT1 induces serine auxotrophy and sensitivity to dietary serine starvation in luminal breast tumors. *Cell Reports*. 2022 Jan 18;38(3):110278. doi: 10.1016/j.celrep.2021.110278. PMID: 35045283. PMCID: PMC8845302.

Complete List of Published Work in Pubmed:
https://www.ncbi.nlm.nih.gov/myncbi/1x9Psf5m1D0Am/bibliography/public/

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: Muir, Alexander

eRA COMMONS USER NAME (credential, e.g., agency login): ALEXMUIR

POSITION TITLE: Assistant Professor

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
University of Chicago	BA/BA	06/2009	Biological Sciences and Romance Languages
University of California, Berkeley	PHD	05/2015	Molecular and Cell Biology
Massachusetts Institute of Technology	Postdoctoral	12/2018	Tumor metabolism

A. Personal Statement

Altered metabolism is a hallmark of cancer. There have been numerous important discoveries regarding how oncogenes alter metabolism to support tumorigenicity and how some metabolic genes can themselves be oncogenes. However, cancer metabolism is not only dictated by cell-autonomous factors but also by cell non-autonomous or microenvironmental factors. Throughout my career, I have used biochemistry and genetic approaches to study how cells from micro-organisms to cancer cells alter metabolism to adapt to such changing environments. This includes key findings from my postdoctoral work that nutrient or metabolic substrate availability is a key microenvironmental regulator of the metabolic pathways used and required by cancer cells, and can dramatically change how cancer cells respond to different metabolic inhibitors. My independent research group continues to study cancer cell metabolic adaptation to the tumor microenvironment. Given my findings that nutrient availability is a key regulator of cancer cell metabolism and therapy response, my group has focused on developing: (1) state-of-the-art quantitative metabolomics techniques to measure with quantitative precision the metabolic substrate microenvironment of pancreatic ductal adenocarcinoma (PDAC) tumors, and (2) novel *ex vivo* models of cancer that mimic the PDAC tumor nutrient microenvironment to elucidate how microenvironmental nutrition impact cancer cell metabolism. My short to mid-term goals are to use these new measurements and model systems to understand how the tumor metabolic microenvironment alters the phenotype and behavior of cancer cells. My long-term goal is to bring to bear increasingly advanced analytical technologies (proteomics, metallomics etc.) to observe the prevailing conditions in the tumor microenvironment, and design model systems to rigorously study how these microenvironmental factors influence cancer and stromal cell biology. I believe this marriage of tumor physiology with cancer cell biology will be critical to understanding with mechanistic rigor how the tumor microenvironment impacts tumor progress, and developing therapies able to target cells reprogrammed by such conditions.

My group's unique expertise in being able to both measure and model nutrient availability in solid tumors allowed us to make the key preliminary finding that microenvironmental nutrient availability is a key factor that limits chemotherapy response in PDAC. We have initiated an already productive collaboration with Dr. Coloff's group that has made progress in understanding the nutrient conditions of TNBC tumors. The combination of our expertise in TME biology and modeling and Dr. Coloff's expertise in TNBC and identifying drug:nutrient interactions uniquely position us to both find novel microenvironment-potentiated therapies for TNBC patients as well as identify nutritional approaches to improving existing therapies.

Ongoing and recently completed projects that I would like to highlight include:

Pancreatic Cancer Action Network Career Development Award
PI: Muir
09/01/2020-08/31/2022
Microenvironment induced metabolic requirements of pancreatic cancers

National Pancreas Foundation Research Grant
PI: Muir
06/01/2020-05/31/2021
Identifying adaptations to metabolic stress in the pancreatic cancer microenvironment

Cancer Research Foundation Young Investigator Award
PI: Muir
04/01/2020-03/31/2022
Regulation of cancer cells and immune cells by the tumor nutrient microenvironment

Brinson Foundation Junior Investigator Award
PI: Muir
12/01/2019-11/30/2022
Identifying the Nutritional and Metabolic Requirements of Pancreatic Cancer

Citations:

1. Apiz Saab JJ, Dzierozynski LD, Jonker PB, Zhu Z, Chen RN, Oh M, Sheehan C, Macleod KF, Weber CR, Muir A (2022) Pancreatic tumors activate arginine biosynthesis to adapt to myeloid-driven amino acid stress. *bioRxiv* 2022.06.21.497008 doi:10.1101/2022.06.21.497008.
2. Sullivan MR, Danai LV, Lewis CA, Chan SH, Gui DY, Kunchok T, Dennstedt EA, Vander Heiden MG‡, Muir A‡. Quantification of microenvironmental metabolites in murine cancers reveals determinants of tumor nutrient availability. eLife. 2019;8:e44235, (2019); PubMed Central PMCID: PMC6510537.
3. Muir A, Vander Heiden MG. The nutrient environment affects therapy. Science. 360(6392), (2018); PubMed Central PMCID: PMC6368963
4. Muir A*‡, Danai LV*, Vander Heiden MG‡. (2018) Microenvironmental regulation of cancer cell metabolism: implications for experimental design and translational studies. Disease Models & Mechanisms. dmm035758. PubMed Central PMCID: PMC6124553

B. Positions, Scientific Appointments, and Honors

Positions and Scientific Appointments

2019-	Assistant Professor, University of Chicago
2015-2018	Postdoctoral Fellow, M.I.T.
2009-2015	Graduate Student, University of California, Berkeley
2008-2009	Physical and Chemical Biology Fellow, University of Chicago
2007	Summer Research Fellow, University of Chicago

Honors

2021	New York Academy of Sciences Cancer and Metabolism Rising Stars Invitee
2019	Halocures Top 40 Under 40 Scientists Award
2018	University of Utah Rising Stars Symposium invitee
2016-2019	NIH Ruth L. Kirschstein National Research Service Award Fellowship
2013-2014	Univ. of California, Cancer Research Committee Graduate Student Fellowship
2013	Biochemistry, Biophysics and Structural Biology Symposium Poster Award. University of California, Berkeley.
2010, 2011	Honorable Mention, NSF Graduate Research Fellowship
2009	*Phi Beta Kappa*, Univ. of Chicago, Chicago, IL
2008-2009	NIH Physical and Chemical Biology Fellowship

2007	University of Chicago Biological Sciences Summer Fellowship
2005-2009	Samsung American Legion National Scholarship
2005-2009	National Merit Scholarship
2005-2009	Robert C. Byrd Fellowship
2005-2009	Mayo Clinic Undergraduate Scholarship

C. Contributions to Science

1. Target of rapamycin complex 2 (TORC2) signaling and membrane homeostasis

As a graduate student in the laboratory of Dr. Jeremy Thorner, I worked to understand how cells sense changes in environment and alter cellular processes to adapt. I focused on understanding the molecular mechanisms by which a signaling pathway initiated by the Target of rapamycin complex 2 (TORC2) kinase mediates cellular resistance to membrane stresses such as hyperosmotic shock. While TORC1 has been studied in detail to elucidate how this network senses nutrients and alters processes to promote growth, little was known about TORC2 beyond its requirement for cellular adaptation to membrane stress. To study how TORC2 mediated cellular resistance to membrane stress, I developed a novel generalizable genetic screening modality to identify functionally important kinase substrates. I used this system to identify novel substrates of the TORC2 signaling network, and found that TORC2 alters sphingolipid, sterol and glycerol metabolism. These TORC2- driven metabolic alterations are required for cellular adaptation to membrane stress, thus defining molecular mechanisms by which TORC2 mediates survival of membrane stress. Additionally, I found that TORC2 is important for adaptation to high osmolarity and acts independently of well-characterized MAPK pathways that eukaryotic cells also use to respond to changes in osmolarity, defining a new osmostress sensing pathway.

a. Roelants FM*, Breslow DK*, **Muir A**, Weissman JS, Thorner J. (2011) Protein kinase Ypk1 phosphorylates regulatory proteins Orm1 and Orm2 to control sphingolipid homeostasis in Saccharomyces cerevisiae. Proc Natl Acad Sci USA. 108(48); PubMed Central PMCID: PMC3228448
Article recommended in F1000: http://f1000.com/prime/13471975
b. **Muir A**, Ramachandran S, Roelants FM, Timmons G, Thorner J. (2014) TORC2-dependent protein kinase Ypk1 phosphorylates ceramide synthase to stimulate synthesis of complex sphingolipids. eLife. e03779; PubMed Central PMCID: PMC4217029
Article recommended in F1000: http://f1000.com/prime/719129839
c. **Muir A**, Roelants FM, Timmons G, Leskoske KL, Thorner J. (2015) Downregulation of TORC2-Ypk1 signaling promotes MAPK-independent survival under hyperosmotic stress. eLife. e09336; PubMed Central PMCID: PMC4552222
d. Roelants FM, Chauhan N, **Muir A**, Davis JC, Menon AK, Levine TP, Thorner J. (2018) TOR Complex 2-regulated protein kinase Ypk1 controls sterol distribution by inhibiting StARkin domain-containing proteins located at plasma membrane-endoplasmic reticulum contact sites. Mol Biol Cell. 29(17); PubMed Central PMCID: PMC6232965

2. Microenvironmental regulation of cancer cell metabolism

As a postdoctoral fellow in Matthew Vander Heiden's lab, my work focused on understanding how the microenvironmental contexts of tumors affects the metabolism and metabolic liabilities of cancer cells. I focused on the microenvironment as a regulator of tumor metabolism as myself and others observed that lung and pancreatic cancer cells in culture require the glutamine catabolism metabolic pathway for survival and proliferation, but that this metabolic pathway was less active and not required for growth of the same lung and pancreatic cancer cells in animal tumor models. This suggested that the tumor microenvironment (TME) is critical for determining how cancer cell metabolism functions and what pathways are amendable to therapeutic targeting. To identify how the TME rewires glutamine metabolism, I built cell culture models where lung cancer cells were cultured under more physiological conditions and found that differences in levels of nutrients, in particular, levels of the amino acid cystine between culture and animal models of lung cancer explain the difference in metabolism of lung cancer cells in these two environmental settings. Further, I found that cystine levels dictate glutamine dependence via the cystine/glutamate antiporter xCT. Thus, xCT expression, in conjunction with environmental cystine, is necessary and sufficient to increase glutamine catabolism, defining important determinants of glutamine metabolism and dependence in cancer. I also initiated a collaboration with Thales Papagiannakopolous' research group to show that lung cancer cells that overexpress xCT due to mutation of the tumor suppressor Keap1 are sensitive to inhibition glutamine catabolism, defining a subset of lung cancer patients that may derive benefit from clinically available glutaminase inhibitors. Together this work has motivated ongoing clinical trials to target glutamine catabolism in Keap1 mutated and xCT-high tumors.

a. **Muir A**, Danai LV, Gui DY, Waingarten CY, Lewis CA, Vander Heiden MG. (2017) Environmental cystine drives glutamine anaplerosis and sensitizes cancer cells to glutaminase inhibition. *eLife*. e27713; PubMed

Central PMCID: PMC5589418

b. Sayin VI*, LeBoeuf SE*, Singh SX, Davidson SM, Biancur D, Guzelhan BS, Alvarez SW, Wu WL, Karakousi TR, Zavitsanou A-M, Ubriaco J, **Muir A**, Karagiannis D, Morris PJ, Thomas CJ, Possemato R, Vander Heiden MG, Papagiannakopoulos T. (2017) Activation of the NRF2 antioxidant program generates an imbalance in central carbon metabolism in cancer. *eLife*. e28083; PubMed Central PMCID: PMC5624783
c. **Muir A***‡, Danai LV*, Vander Heiden MG‡. (2018) Microenvironmental regulation of cancer cell metabolism: implications for experimental design and translational studies. *Disease Models & Mechanisms*. dmm035758; PubMed Central PMCID: PMC6124553
** indicates equal contribution*
‡ indicates corresponding authors

3. Defining the metabolic microenvironment of tumors

Given my postdoctoral work highlighting the critical importance of the tumor microenvironment in dictating tumor metabolism, my independent laboratory has focused on defining the metabolic microenvironment of tumors to enable future studies delineating how the tumor microenvironment influences cancer metabolism. We have begun these studies by developing protocols and a quantitative metabolomics platform to extract interstitial fluid (the perfusate that carries nutrients to tissues and solid tumors) from tumors and then measure the concentrations of 150+ critical metabolites and nutrients in the microenvironment of different tumor types. This work provides an atlas of nutrients that cancer cells could engage to support tumor progression and is the first study to provide quantitative insight into nutrient stresses in the tumor microenvironment. We have endeavored to make these tools and protocols available and they have become broadly used by the tumor metabolism community. Our group has made participated in several collaborations using these tools to answer questions related to tumor-stroma nutrient sharing in the tumor microenvironment. Using our atlas of tumor nutrition to determine how cancer cell metabolism adapts to microenvironmental nutrient levels and opens new therapeutic targets is the current focus of my laboratories research.

a. Sullivan MR, Danai LV, Lewis CA, Chan SH, Gui DY, Kunchok T, Dennstedt EA, Vander Heiden MG‡, **Muir A**‡. (2019) Quantification of microenvironmental metabolites in murine cancers reveals determinants of tumor nutrient availability. *eLife*. e44235; PubMed Central PMCID: PMC6510537
‡ indicates corresponding authors
Covered by MIT News
Covered by The Atlantic
Top 5% Altmetric score
b. Sullivan MR, Lewis CA, **Muir A.** (2019) Isolation and quantification of metabolite levels in murine tumor interstitial fluid by LC/MS. *Bio-protocol*. 9(22); PubMed Central PMCID: PMC7853983
c. Francescone R, Vendramini-Costa DB, Franco-Barraza J, Wagner J, **Muir A**, Gabitova L, Pazina T, Luong T, Shah N, Rollins D, Gupta S, Roshan T, Restifo D, Lau A, Zhou Y, Cai KQ, Hensley H, Nicolas E, Kruger W, Devarajan K, Balachandran S, El-Deiry WS, Vander Heiden MG, Campbell KS, Astsaturov I, Cukierman E (2020). Netrin G1 promotes pancreatic tumorigenesis through cancer associated fibroblast driven nutritional support and immunosuppression. *Cancer Discovery(* 2021)11(2):446-479; Epub 2020 doi: 10.1158/2159-8290.CD-20-0775; PubMed Central PMCID: PMC7858242
d. Reinfeld BI, Madden MZ, Wolf MM, Chytil A, Bader JE, Patterson AR, Cohen AS, Ali A, Do BT, Lewis CA, **Muir A**, Hongo RA, Young KL, Brown RE, Todd VM, Huffstater T, Abraham A, O'Neil RT, Wilson MT, Xin F, Tantawy MN, Merryman WD, Johnson RW, Williams CS, Mason EF, Mason FM, Beckermann KE, Vander Heiden MG, Manning HC, Rathmell JC, Rathmell WK (2021). Cell Programmed Nutrient Partitioning in the Tumor Microenvironment. *Nature*. 238428; PubMed Central PMCID: PMC8122068

4. Tumor microenvironmental nutrient availability as a key regulator of cancer cell phenotypes

After developing tools to measure nutrient availability in the tumor microenvironment, my research team has endeavored to understand how such tumor physiology impacts cancer cell biology and phenotypes. We have built custom cell culture models that recapitulate the tumor nutrient environment and using these models found that nutrient availability in tumors is a key microenvironmental factor that drives many behaviors of cancer cells that are observed only in the tumor microenvironment. As these models provide access to cancer cell phenotypes normally only seen in complex tumors, they have become key tools used by the PDAC field and are in use by over 20 research labs across the world. One of the *in vivo* PDAC cell behaviors we have found microenvironmental nutrients drive is profound resistance to chemotherapeutics that is often observed in PDAC tumors, but not in isolated PDAC cells cultured in standard laboratory models. We have used our microenvironmental nutrient mimicking models to understand how tumor physiology rewires PDAC cell biology to enable therapy resistance.

a. Apiz Saab JJ, Dzierozynski LD, Jonker PB, Zhu Z, Chen RN, Oh M, Sheehan C, Macleod KF, Weber CR, **Muir A** (2022) Pancreatic tumors activate arginine biosynthesis to adapt to myeloid-driven amino acid stress. *bioRxiv* 2022.06.21.497008 doi:10.1101/2022.06.21.497008.
   Top 5% Altmetric score
b. Sela Y, Li J, Maheswaran S, Norgard RJ, Yuan S, Hubbi ME, Doepner M, Xu JP, Ho ES, Mesaros C, Sheehan C, Croley G, **Muir A,** Blair IA, Ophir S, Dang CV, Stanger BZ (2022) Bcl-xL enforces a slow-cycling state necessary for survival in the nutrient-deprived microenvironment of pancreatic cancer. *Cancer Res.* Epub 2022 doi: 10.1158/0008-5472.CAN-22-0431. PubMed Central PMID: 35315913
c. **Muir A**, Vander Heiden MG. (2018) The nutrient environment affects therapy. *Science*. 360(6392); PubMed Central PMCID: PMC6368963

Complete List of Published Work in MyBibliography:
https://www.ncbi.nlm.nih.gov/sites/myncbi/alexander.muir.1/bibliography/49866069/public/?sort=date&direction=ascending

DEPARTMENT OF BIOMEDICAL GENETICS

Isaac S. Harris, Ph.D.
Assistant Professor of Biomedical Genetics

November 17, 2022

To: CBC Catalyst Award Review Panel Members
RE: Collaboration with Drs. Coloff and Muir

Review Panel Members,

I am writing to express my utmost enthusiasm and excitement for Dr. Coloff and Dr. Muir's CBC Catalyst Award application, "Improving Triple-Negative Breast Cancer Therapy by Targeting the Tumor Metabolic Microenvironment". I have enjoyed productive collaborations with Jon Coloff and Alex Muir for several years, and I look forward to working together in the coming years. Their goal of identifying and exploiting novel metabolic dependencies in triple-negative breast cancer (TNBC) is an exciting area of investigation with important translational implications.

I am an Assistant Professor in the Department of Biomedical Genetics at the University of Rochester Medical Center and the Wilmot Cancer Institute. My lab has expertise in using high-throughput technology to better understand novel vulnerabilities in cancers. In particular, we have pioneered a platform that uses small molecule libraries to identify and characterize novel tumor cell phenotypes. The development of this platform has led to several important publications, some that were published in collaboration with Jon Coloff (Harris et al., 2019, Cell Metabolism, https://doi.org/10.1016/j.cmet.2019.01.020; Nicholson et al., 2019, Science Signaling, https://doi.org/10.1126/scisignal.aay0482; Shu et al., 2020, Molecular Cell, https://doi.org/10.1016/j.molcel.2020.04.027; Drijvers et al., 2021, Cancer Immunology Research, https://doi.org/10.1158/2326-6066.CIR-20-0384; Koduri et al., 2021, Science Advances, https://doi.org/10.1126/sciadv.abd6263; Shi et al., 2022, Cancer Cell, https://doi.org/10.1016/j.ccell.2022.07.011). We have continued to collaborate with Jon and recently assisted him to identify that the sensitivity of triple-negative breast cancer cells to 6-mercaptopurine and lometrexol is dependent on the levels of hypoxanthine in tissue culture media. An outstanding question is how the nutrient levels found in actual breast tumors can affect the sensitivity of TNBC cells to cancer therapeutics. To address this issue, I am delighted to work with Jon and Alex to elucidate compounds that are affected by the culture in the breast tumor-specific media they are developing. This research goal not only leverages upon our established high-throughput pharmacologic platform but builds directly on our longstanding (and fruitful) collaborations.

In closing, I offer my full support for Jon and Alex's proposed collaborative studies and for their CBC Catalyst Award application. The questions their research proposal addresses are compelling, and I look forward to helping them investigate the translational potential of manipulating nutrient levels in breast tumors to improve the efficacy of neoadjuvant chemotherapy.

Sincerely,

Dr. Isaac Harris
Assistant Professor
Department of Biomedical Genetics
University of Rochester Medical Center
Wilmot Cancer Institute
isaac_harris@urmc.rochester.edu

601 Elmwood Avenue • Box 633 • Rochester, NY 14642
585-273-43653 • 585-273-1450 fax • Isaac_Harris@urmc.rochester.edu